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Gel Extraction Kit

Product Number: DRK0101

Shipping and Storage

1. All solutions should be clear. If the ambient temperature is low, the solution may form precipitates and should not be used directly. It can be heated in a 37℃ water bath for a few minutes to restore clarity. It should be restored to room temperature before use.
2. Storing at low temperatures (4℃ or -20℃) can cause solution precipitation, affecting the effectiveness of use. Therefore, transportation and storage are carried out at room temperature (15℃ -25℃).
3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.

Components

Component	Storage	DRK0101 50 Preps	DRK0102 100 Preps	DRK0103 200 Preps
Balance Buffer	RT	5 ml	10 ml	20 ml
Buffer DD	RT	40 ml	75 ml	150 ml
Buffer WB	RT	13 ml	25 ml	50 ml
Buffer EB	RT	10 ml	15 ml	15 ml
Adsorption column EC	RT	50	100	200
Collection tube (2ml)	RT	50	100	200

Description

In the presence of high dissociation salts, DNA fragments selectively adsorb onto the silica matrix membrane inside the centrifuge column. Through a series of rapid rinsing centrifugation steps, Buffer WB removes impurities such as primers, nucleotides, proteins, enzymes, etc. Finally, low salt, high pH Buffer EB elutes pure DNA from the silica matrix membrane.

Features

1. The silicon matrix membranes inside the centrifugal adsorption column are all made of specially designed adsorption membranes, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. High quality buffer DD was used, which does not contain traditional buffer DD's sodium iodide and perchlorate, and does not inhibit downstream reactions such as enzyme digestion and cloning after recovery.
3. Buffer DD is modulated with phenol red to become a yellow color, which facilitates observation of sol effect and monitoring of pH changes to achieve optimal binding effect, greatly improving recovery efficiency.
4. The improved Buffer DD formula greatly enhances buffering capacity and stability, allowing pH to be buffered within the optimal binding range even with significant sample changes.
5. Fast and convenient, without the need for toxic reagents such as phenol and chloroform, and without the need for ethanol precipitation.

Application

Suitable for DNA recovery from agarose gel

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to

13000rpm.

2. Buffer DD contains irritating compounds, and latex gloves should be worn during operation to avoid contact with skin, eyes, and clothing. If it gets on the skin or eyes, immediately rinse with plenty of water or physiological saline.
3. The recovered and purified DNA fragments are generally between 100bp and 40kb, and the recovery efficiency of long and short fragments rapidly decreases.
4. The amount of recovered DNA is related to the amount of starting DNA, elution volume, and DNA fragment size. Generally, DNA fragments ranging from 1-15ug to 100bp-5kb can have a recovery rate of up to 85%.
5. When cutting and recycling, UV light observation can damage DNA fragments. Low energy long wave ultraviolet light should be used as much as possible, and the processing time under ultraviolet light should be shortened as much as possible.
6. Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, linking, and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5, as low pH can affect elution efficiency. Wash with water, DNA fragments should be stored at -20℃. If DNA fragments need to be stored for a long time, they can be eluted with TE buffer (10mM Tris HCl, 1mM EDTA, pH 8.0), but EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

Use of balance buffer

1. Description

During the long-term placement of the nucleic acid adsorption silica gel membrane column, it reacts with the charge/dust in the air, affecting its nucleic acid binding ability. After pre-treatment with Balance Buffer, the hydrophobic groups of the silica gel membrane in the column can be greatly reduced, improving the binding ability of nucleic acids. Thereby improving the recovery efficiency or yield of silicone columns. Balance Buffer is a highly alkaline solution. If accidentally touched, please clean with plenty of tap water. After use, it is necessary to tighten the bottle cap to avoid contact with air. Store at room temperature. During storage, precipitation may occur. Please heat to 37 ℃ to completely eliminate the precipitation.

2. Protocol

Take a new silicone membrane adsorption column and place it in a collection tube. Take 100μl of Balance Buffer and transfer it into the column. Centrifuge at 13000rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube. At this point, the Balance Buffer has completed preprocessing the columns. Follow the subsequent operating steps.

Protocol

Tip: Before using it for the first time, please add the specified amount of anhydrous ethanol to the Buffer WB. After adding it, please mark the ethanol added in a timely manner to avoid adding it multiple times!

1. Under the long wave ultraviolet light, use a clean blade to cut off the DNA strips to be recovered, and try to cut off the DNA free gel to get the smaller the gel volume, the better.
2. Put the cut gel containing DNA strips into a 1.5ml centrifuge tube and weigh it.

   First weigh an empty 1.5ml centrifuge tube, then put in a gel block and weigh again. Subtract the two weights to get the weight of gel.
3. Add 3 times the volume of Buffer DD.

   If the gel weighs 100mg and its volume can be regarded as 100μl, 300μl Buffer DD shall be added.

   If the gel concentration is greater than 2%, 6 times the volume of Buffer DD shall be added.
4. Place in a 56℃ water bath for 10 minutes (or until the glue is completely dissolved). Vortex oscillation every 2-3 minutes helps accelerate dissolution.

   Optional, generally unnecessary: add 150μl isopropyl alcohol per 100mg of initial gel weight, shake and mix well.

   Sometimes adding isopropanol can improve the recovery rate, do not centrifuge after adding. When recovering fragments larger than 4Kb, not adding isopropanol may sometimes reduce the recovery efficiency.

   Balance Buffer pre-treatment adsorption column:The pre-treatment of silica gel membrane adsorption column using

Balance Buffer is a necessary step. For specific methods, please refer to the previous section "Use of balance buffer"

5. Add the solution obtained from the previous step to the adsorption column EC (the adsorption column is placed in the collection tube), let it stand at room temperature for 1 minute, centrifuge at 12000 rpm for 30-60 seconds, and discard the waste liquid in the collection tube.

   If the total volume exceeds 750µl, the solution can be added twice to the same adsorption column EC.

   After mixing the filtered Buffer DD with the remaining strong alkaline Balance Buffer in the collection tube, the Buffer DD may change from yellow to orange red or even purple, which is the normal color change of the phenol red pH indicator under alkaline conditions.
6. Add 600µl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
7. Add 600µl Buffer WB, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.
8. Put the adsorption column EC back into the empty collection tube, centrifuge at 12000 rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.
9. Take out the adsorption column EC and place it in a clean centrifuge tube. Add 50µl of Buffer EB to the middle of the adsorption membrane (Buffer EB is better heated in a 65-70℃ water bath beforehand). Leave at room temperature for 2 minutes and centrifuge at 12000 rpm for 1 minute. If a large amount of DNA is required, the obtained solution can be re added to the adsorption column and centrifuged for 1 minute.

   The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 25µl. If the volume is too small, it will reduce the DNA elution efficiency and yield.